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Investigation on lactation persistency and *IGF-I* gene polymorphisms in dairy sheep

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ABSTRACT

Insulin-like growth factor I (IGF-I) plays an important role in mammary growth and function. We detected two novel SNPs in the 5′UTR of the *IGF-I* gene: g.855G>C and g.857G>A of locus X69472, and genotyped 103 sheep of three breeds at the identified polymorphisms. The same animals were genotyped at a previously reported SNP (g.271C>T; accession X69473) located in exon 3. From the SNPs we built 4 haplotypes. We calculated lactation persistency from test day records of the genotyped animals as the ratio of milk (kg), fat (g) and protein (g) yield from day 86 to 170 of machine milking to yields during the first 85 days of machine milking. We estimated allele substitution effect on persistency for each SNP and haplotype. We found that allele T of SNP g.271C>T and haplotype [G;T] had a positive effect on maintaining a constant yield level during lactation. The improvement of lactation persistency could be highly beneficial to the sustainable use of local breeds.

1. Introduction

Haenlein (2001) wrote that sheep milk yields can differ more than 10 times between breeds and within breeds, and lactation lengths can vary by 100%; however, sheep breeders and scientists do not seem to have considered the lactation length an important breeding goal. Where breeding programs and milk recording systems are implemented, dairy sheep are selected on the basis of milk yield, fat and protein content (Astruc et al., 2005). Milk yield is evaluated according to a reference length of the lactation, ranging from 120 to 195 days, which is arbitrarily defined for each breed (Astruc et al., 2005). Using milk yield adjusted for lactation length as a selection criterion does not fully exploit the within breed variation for days in milk.

The dramatic shrinking of sheep genetic resources requires novel approaches in the evaluation of the breeds. Forty five years ago, the Gentile di Puglia and Altamurana local breeds were found in large numbers in southeastern Italy with a population of approximately 1 million head for each breed. Their population has decreased substantially since then, and they have been replaced with the Sarda breed which has increased from approximately 2.5 million head in 1963 to over 5 million head in 2000. A major reason for the increase in Sarda compared to the local breeds is the high milk production of the Sarda which has increased 30% in recent years (AIA, 1981–2005). Although milk of the two local breeds is of better quality for dairy processing (Signorelli et al., 2008), with fat and protein content generally 4–5% higher than in the Sarda, this better quality is not sufficient to compensate the poorer yield. However, more than the daily milk yield, the major penalizing factor lies on the days in milk, that are averagely 30–40% less in the Gentile di Puglia and Altamurana compared to the Sarda.

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Persistency of lactation yield is an important element of total yield; cows are persistent if they tend to maintain their peak yield within a lactation period (Grossman et al., 1999). Capuco et al. (2003) showed that increased milk yield during early lactation appeared mainly due to increased secretory activity per cell; on the other hand, the decline in milk yield with advancing lactation was solely due to decreased cell number. The action of the mammogenic and lactogenic hormones: prolactin (Prl), growth hormone (GH), and of the insulin-like growth factor I (IGF-I), are considered to explain the essentials of mammary growth and function (Akers, 2002). Weber et al. (2000) showed the importance of the local synthesis of IGF-I and IGF binding proteins in the mammary tissue in mediating the effects of somatotropin treatment and feeding level on the development of mammary gland. In a recent work, Akers (2006) gives an overview of the actions of the mammogenic and lactogenic hormones and their signaling cascades, and emphasizes that there is an exciting universe of growth factors, transcription factors, receptors, intracellular signaling intermediates, and extracellular molecules that must ultimately interact to determine the functional capacity of mammary gland in the lactating cow. Although most of the literature relevant to mammary cell regulation is devoted to the dairy cow, a few studies showed interest in the ovine *IGF-I* gene (Wong et al., 1989; Dickson et al., 1991; Pell et al., 1993; Ohlsen et al., 1993; Shen et al., 2005) and showed that, in mammals, exons 1 and 2 are differentially spliced to exon 3 producing alternate class 1 and class 2 transcripts. In sheep, there is evidence for a third leader sequence which is spliced on to exon 3. This could indicate an additional exon or a differential splicing event from an extended exon, referred as exon 1W after Wong et al. (1989).

Purpose of the present study is to search for polymorphisms in the *IGF-I* gene in sheep of different breeds and to test their influence on lactation persistency, in order to set up appropriate genetic improvement programmes and to propose possible clues for the sustainable use of the endangered sheep breeds.

2. Materials and methods

2.1. Animal recording

The study was conducted on milk records of 103 sheep of three breeds: 33 Gentile di Puglia, 36 Altamurana and 34 Sarda, raised in the same flock with traditional management system, consisting of lambing in November, suckling for 35–40 days, then regular machine milking of the ewes twice a day. Adult weight of the ewes of the three breeds was similar, ranging between 40 and 45 kg. Milking ewes were grazed natural pasture with feeding supplement in the shed of 250 g pellet concentrate, 150 g oat grains and 1.5 kg oat and vetch hay. Milk recording was performed every second week along the lactation, on the same day for all the ewes, following the regulation of the International Committee for Animal Recording (ICAR, 2009). A total of 889 records were so obtained.

2.2. Genotyping

Three amplicons of 567 bp and 459 bp were obtained by PCR amplification of the DNA of the 103 sheep. The first amplicon included the DNA fragment from 809 to 1375 base of locus X69472 (Dickson et al., 1991) and covered *IGF-I* exon 1W, including part of the 5′UTR; the second amplicon from 1265 to 1723 base of the same locus, covering the whole exon 1. The two overlapping amplicons covered a DNA region of 915 bp. No SNP had previously been reported in this region. The third amplicon (264 bp) included 43–306 base of locus X69473 (264 bp) encoding exon 3, where SNP (g.271C>T) had already been reported (Pariset et al., 2006).

Primers used for the amplification were as follows:

Amplicon 1 – forward primer: tgagatcattcccctcacttg; reverse primer: gcaggctctatctgctctgaa;
Amplicon 2 – forward primer: cctgtctacagtgtctgtgttttg; reverse primer: tttcagatcccacagaattgc;
Amplicon 3 – forward primer: cacacacctt gttgcactcc; reverse primer: agagcatccaccaactcagc.

Direct sequencing was performed for all samples on a Perkin Elmer ABI Prism 310 DNA sequencer. The PCR for sequencing was obtained by using ABI Prism BigDye Terminator Cycle Sequencing, Ready Reaction Kits (version 1.1 – Applied Biosystems). The protocol for Single and Double Stranded DNA was optimized in 20 μl of final volume, containing: 4 μl of Terminator Ready Reaction mix, 10–15 ng PCR product and 5 pmol of single primer. The product of sequencing reaction was purified with Nucleoseq kit (M-Medical).

Haplotypes were constructed using Arlequin software (Excoffier et al., 2005).

An *in silico* analysis of the fragment containing the two amplicons was performed with Genomatix software (Genomatix, 1998–2008) to verify the presence of putative binding sites of transcription factors.

2.3. Statistical analysis

Lactation persistency was calculated as the ratio of milk (kg), fat (g), and protein (g) yields from day 86 to 170 of machine milking to yields during the first 85 days of machine milking. The effect of the Single Nucleotide Polimorphisms (SNPs) on lactation persistency (milk, fat and protein yield) was estimated by regressing the phenotype on the number of copies of one allele of each SNP (Sherman et al., 2008) or haplotype, using GLM procedure of SAS software (SAS, 2007), with the following model:

$$Y_{ijkl} = \mu + B_i + T_j + L_k + b_1(D) + b_2(G) + e_{ijkl}$$

where Y_{ijkl} = persistency: milk (kg), fat (g) and protein (g) yield; B_i = fixed effect of the breed; T_j = fixed effect of number of lambs born (1 or 2); L_k = fixed effect of parity (first, second, or third and greater); $b_1(D)$ = covariate of the number of days between lambing and removal of the lamb(s); $b_2(G)$ = covariate of the copy number of each allele (allele substitution effect); e_{ijkl} = residual.

The same model was used to analyze days of total lactation and days of machine milking with the exception that the covariate of the number of days between lambing and removal of the lamb(s) was replaced with the covariate of milk yield at the first test day.

3. Results

Direct sequencing of the 1035 bp DNA region, covering exon 1W and exon 1 of *IGF-I* gene, allowed to detect two novel SNPs: g.855G>C and g.857G>A of locus X69472; on the contrary, no polymorphism was found in the DNA region encompassing exon 1. The novel SNPs fall at 28 and 26 bp 5′ to the alternative exon 1W.

Results of the *in silico* analysis of the fragment containing the novel detected SNPs (g.855G>C and g.857G>A) indicated that they fall in the core sequence (acgaggGGTCatccca**gcg**ccgtct) of the retinoid X receptor heterodimers (*RXR*), member of the superfamily of nuclear factors that includes *VDR* (vitamin D receptor). *RXR* and *VDR* form a heterodimeric complex and bind cooperatively to vitamin D responsive elements (*VDREs*) to activate or repress the transcription of a multitude of genes which regulate a variety of physiological functions (Lemon et al., 1997). Matilainen et al. (2005) identified 15 *VDRE* (candidate vitamin D response elements) within human IGF binding